The Fate of the Initial Follicle Pool: Mathematical Modeling Demonstrates Its Sufficiency for Adult Fertility

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Abstract

The importance of the initial follicle pool for fertility in adult mammals is not fully understood. We developed a mathematical model of the dynamics of early stage follicles (primordial, primary, secondary) to examine the importance of the initial follicle pool as it relates to reproductive senescence. Follicle counts of CD1 mice were fit to a series of first order differential equations with the follicle populations simulated according to two mechanisms: (i) an initial pool of primordial follicles as the only follicle source, (ii) an initial primordial follicle pool supplemented by germline stem cells. The fixed pool model fit the experimental data, capturing the maximum observed in primary follicle number at 4-6 months. Although no germline stem cells could be identified by staining for SSEA-1, we next incorporated the suggested 77 *de novo* follicles per day (1). With this addition, the model no longer fits the observed decreases in follicles with time and does not parallel the accumulation and subsequent reduction in primary follicles during the early fertile lifespan of the mouse. Further simulations indicated that only low rates of generation are potentially consistent with experimental observations (less than 10 *de novo* follicles/day). Our results agree with the concept that the initial endowment of ovarian follicles is not supplemented by an appreciable number of stem cells; rather, it is sufficient to ensure the fertility needs of the adult mouse.

Study Design and Rationale

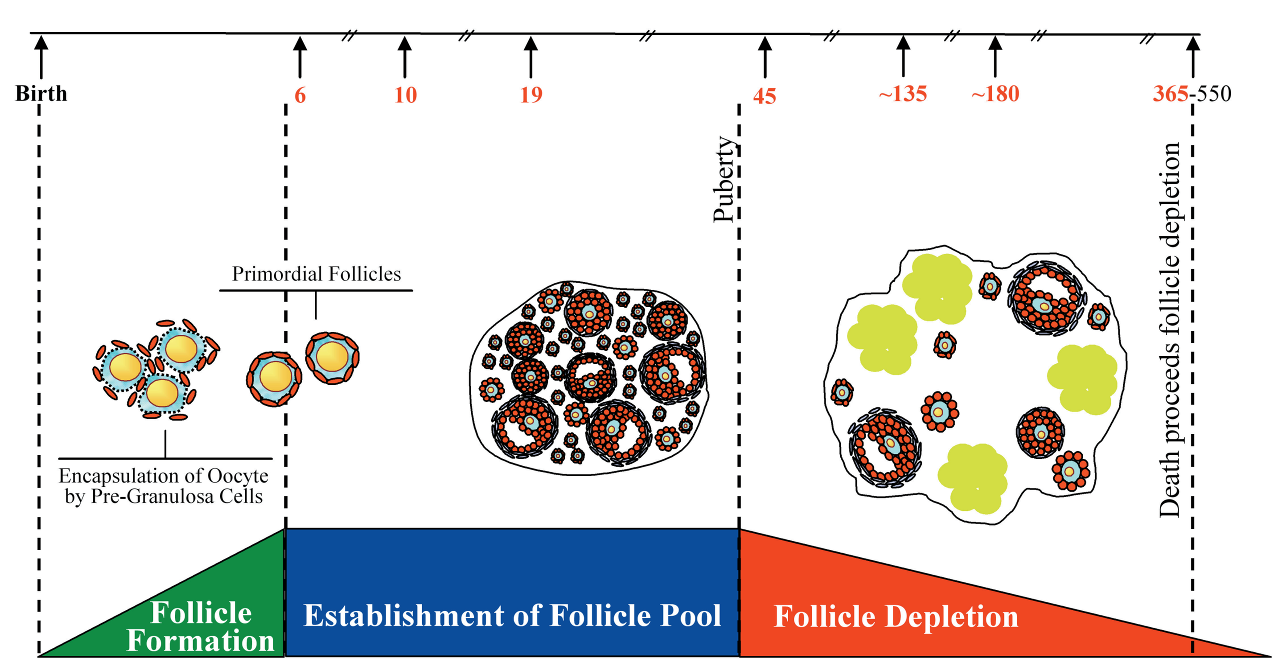
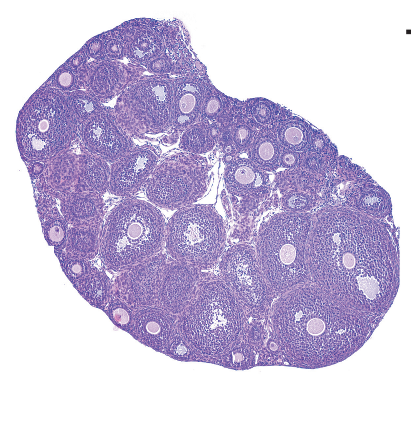


Figure 1. Formation of the initial follicle pool begins shortly after birth and is completed around day 6 in the mouse. Reproductive cyclicity begins at puberty and fertility begins to decline after 1 year. To examine the fate of the initial follicle pool, ovaries were collected and primordial/primary/secondary follicles counted and used to fit parameters in a mathematical model. Red font indicates the days on which follicle counts were acquired for use in the mathematical model.

Ovarian Follicle Counting



Follicle Class	Description
Primordial	1) Small oocyte, surrounded by squamous or squamous/cuboidal granulosa cells 2) All follicles counted; atretic follicles not observed
Primary	1) Small oocyte, surrounded by a single layer of cuboidal granulosa cells 2) All follicles counted; atretic follicles not observed
Secondary	1) Larger oocyte, more than one layer of granulosa cells but no antrum 2) Only counted if nucleolus present; atretic follicles not counted

Figure 2. Ovaries from CD1 mice were serially sectioned (5 μm) and stained. Two or more individuals counted every 5th section. The average number of follicles for each set of ovarian sections was divided by the number of sections, and then multiplied by a normalization factor to account for ovary size and prevent double counting of primordial/primary follicles.

		Normalization		Number of Follicles per Animal		
Age (Days)	(n)	PF/1°	2°	PF	1°	2°
6	11	37.5	75	10265 ± 489	414 ± 29	447 ± 49
10	8	50	100	8662 ± 660	567 ± 82	984 ± 85
19	6	62.5	125	5127 ± 488	294 ± 21	656 ± 74
45 days	4	92.5	185	2706 ± 387	476 ± 52	596 ± 90
4.5 months	4	136	272	1583 ± 81	595 ± 21	531 ± 49
6 months	4	122.5	245	1487 ± 109	595 ± 23	519 ± 55
12 months	4	115	230	477 ± 145	281 ± 67	166 ± 41

Localization of SSEA-1

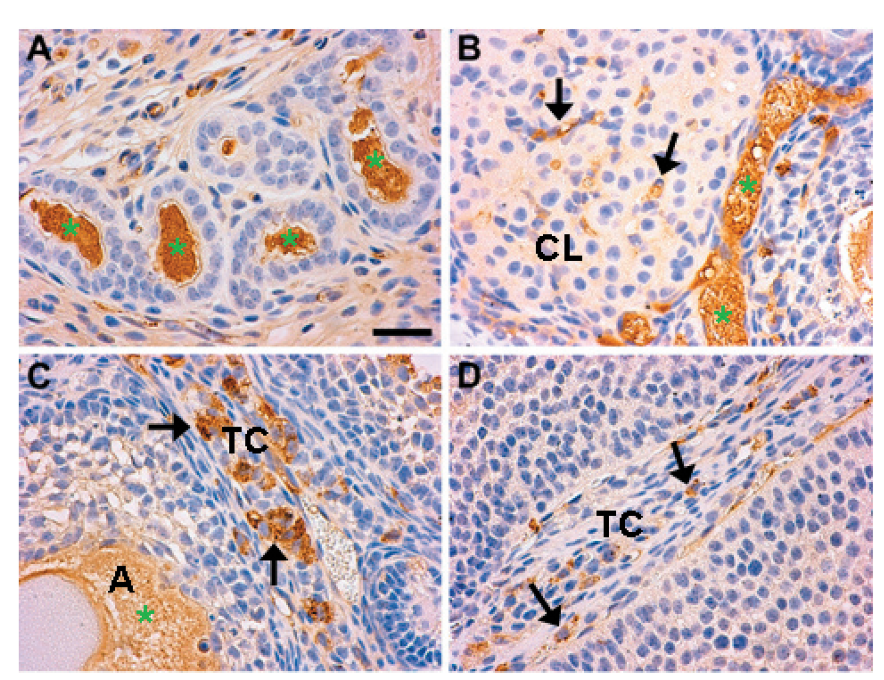
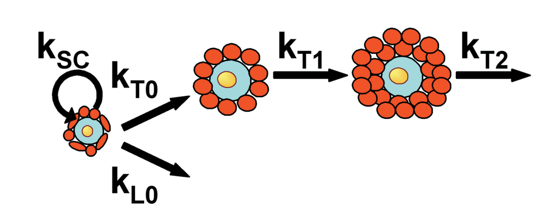


Figure 3. SSEA-1 immunohistochemistry. **(A)** Adult tissue stained for SSEA-1 indicating densely immunoreactive blood vessel lumens within the oviduct. **(B)** SSEA-1 staining within corpora lutea and theca cell layer between the corpora lutea and a follicle. **(C,D)** SSEA-1 staining within antral fluid of an antral follicle and within the theca cell layers between large follicles. (*) indicates staining within large fluid filled areas (blood vessels or antral fluid). Arrows indicate more discrete staining within the theca cell layers or in corpora lutea, also sites of ovarian vasculature in the adult animal. CL = corpora lutea, TC = theca cell, A = antrum. Scale bar; 50μm.

Mathematical Model of Follicle Dynamics



$$\frac{dF_0}{dt} = -k_{T0}F_0 - k_{L0}F_0 + k_{SC}$$

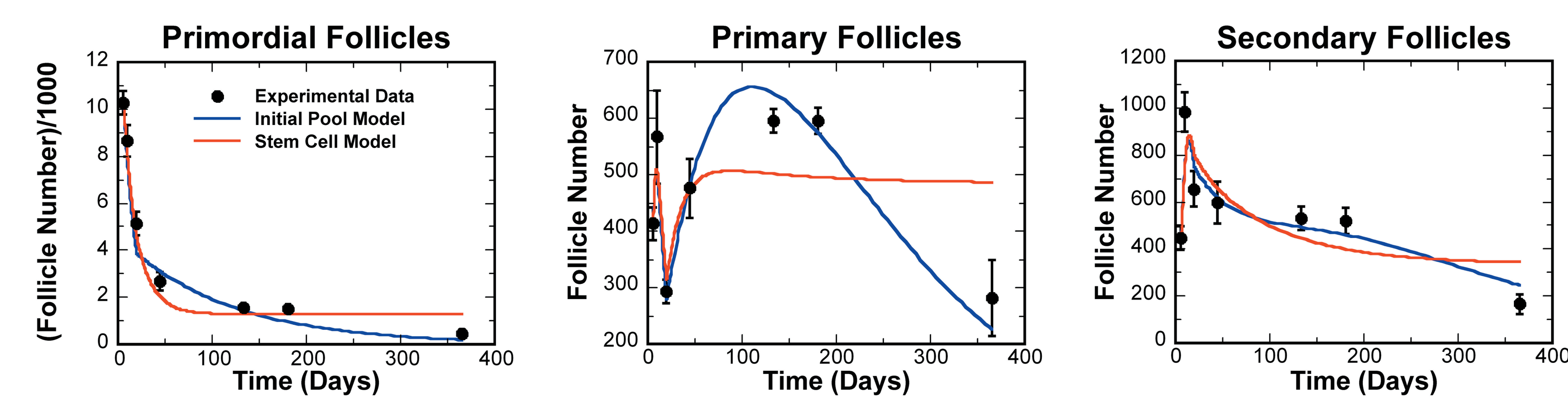
$$\frac{dF_1}{dt} = k_{T0}F_0 - k_{T1}F_1$$

$$\frac{dF_2}{dt} = k_{T1}F_1 - k_{T2}F_2$$

Figure 4. The persistence of primordial (F_0), primary (F_1), and secondary follicles (F_2) was investigated using a mathematical model that describes primordial follicle loss and the transitions between follicle stages. These processes were modeled as first order and solved numerically using Matlab. Germline stem cell generation rates were modeled as a zero-order process based on the recent report of germline stem cells in the adult mouse ovary (1).

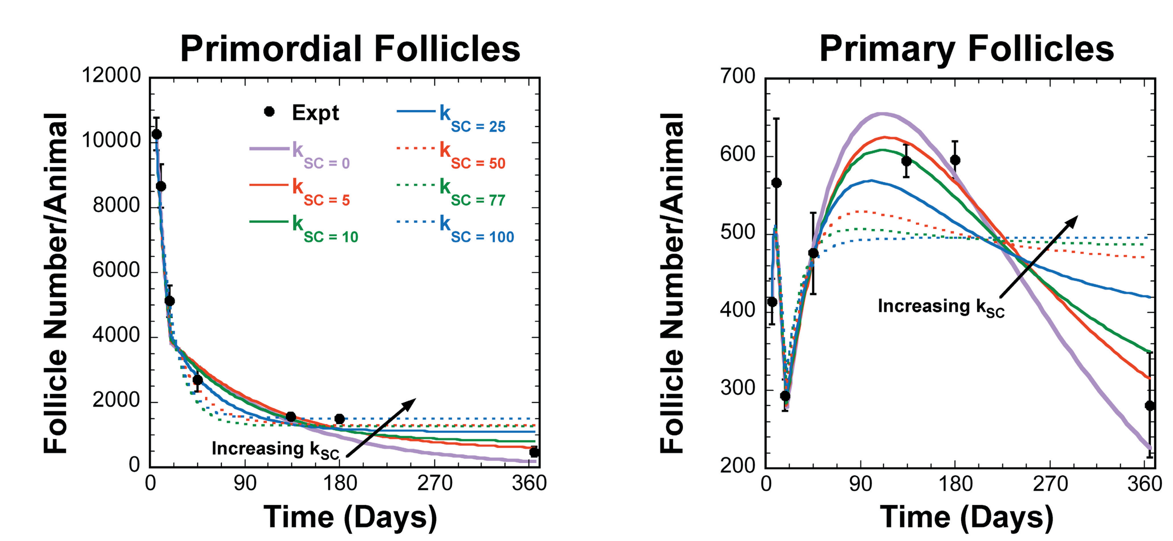
	Fixed Follicle Pool Model		Stem Cell Model	
	Before postnatal day 20	After postnatal day 20	Before postnatal day 20	After postnatal day 20
k_{T0}	0.024 d^{-1}	0.0034 d^{-1}	0.022 d^{-1}	0.0034 d^{-1}
k_{L0}	0.046 d^{-1}	0.0053 d^{-1}	0.046 d^{-1}	0.056 d^{-1}
k_{SC}	---	---	77 F_0/d	77 F_0/d
k_{T1}	0.40 d^{-1}	0.0090 d^{-1}	0.40 d^{-1}	0.0090 d^{-1}
k_{T2}	0.19 d^{-1}	0.013 d^{-1}	0.19 d^{-1}	0.013 d^{-1}

Parameters were solved separately for days 6-20 and days 20-365, coincident with experimentally observed spikes in pituitary FSH as the reproductive axis matures.



- The fixed initial pool model parameters indicate a flux of 3-6 follicles/day, indicating 12-24 follicles per 4-day estrus.
- The stem cell model predicts relatively stable levels of follicles after approximately day 60, as a result of the introduction of *de novo* primordial follicles.

Impact of Stem Cell Kinetic Parameter



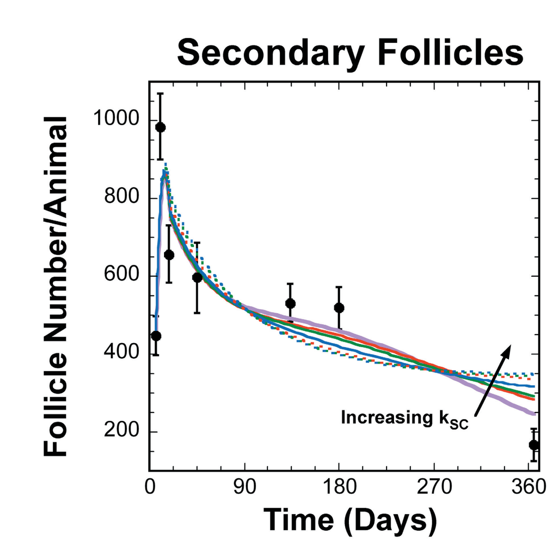


Figure 5. Varying k_{SC} from 0 to 100 *de novo* follicles/day indicated that the addition of *de novo* follicles results in an accumulation of primordial and secondary follicles that was not seen in the experimental data or initial pool model (k_{SC}=0). High levels of k_{SC} did not capture the increase and subsequent reduction in primary follicles that was seen experimentally.

Conclusions

- We were unable to identify germline stem cells in the adult ovary utilizing SSEA-1 as a marker; staining was consistent with the view that SSEA-1 is a marker of stem cells in the blood (2)

- Utilizing a rigorous manual follicle counting procedure, we developed a mathematical model of follicle dynamics which parallels the experimentally observed accumulation and subsequent reduction in primary follicle numbers during the early fertile lifespan; this behavior was not observed in models which incorporated significant levels of germline stem cell activity

Our results agree with the concept that the initial endowment of ovarian follicles is not supplemented by an appreciable number of stem cells; rather, this initial pool is sufficient to ensure the fertility needs of the adult mouse.

References and Acknowledgments

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